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Dinitroaniline Herbicide Cross-Resistance in Resistant *Setaria italica* Lines Selected from Interspecific Cross with *S. viridis*

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Abstract: Breeding of herbicide-resistant foxtail millet (*Setaria italica* (L.) Beauv.) is desirable in modern agriculture because no selective herbicide is available for this crop. Trifluralin-resistant millet lines obtained from a interspecific cross between a resistant green foxtail (*Setaria viridis* (L.) Beauv.) and a foxtail millet cultivar were tested for response to five dinitroaniline herbicides in greenhouse and field experiments. The results in the greenhouse study showed that the resistant type was cross-resistant to all tested herbicides. ED_{50} values indicated that the highest resistance was obtained with trifluralin, the resistant biotype being seven and 33 times more resistant than the parent cultivar at the emergence and seedling stages, respectively. However, the doses recommended for efficient weed control resulted in some detrimental effects on emergence and growth in the field. Strategies for use of this resistance to control weeds in millet fields are proposed.

Key words: foxtail millet, cross-resistance, dinitroaniline herbicides

1 INTRODUCTION

Foxtail millet (*Setaria italica* (L.) Beauv) is an important food crop of China and India (more than 1% of total grain production in China),[1] and is grown in Europe for diet food and bird seed. Weed control has been the main problem limiting further foxtail millet production. Hand weeding is currently carried out as no selective herbicide is available.[2,3] Some weed control may be achieved for the period of crop emergence using bentazone, but only a low dose (300 g ha^{-1}) can be used because of toxicity to the crop. Breeding herbicide-resistant varieties appears to be one way to overcome this problem by allowing use of other herbicides instead of hand weeding. Breeding for herbicide-resistant crops is now a very active research field.[4]

One source of herbicide-resistance genes is the wild gene pool of crops and weeds.[2,5,6] Green foxtail (*Setaria viridis* (L.) Beauv) is a very abundant grass weed worldwide. It is closely related to and presumed to be the wild ancestor of cultivated foxtail millet.[7] Interspecific hybridization has already been used to transfer one herbicide resistance, atrazine resistance, to foxtail millet.[2,8]

Recently, a trifuralin-resistant green foxtail was detected in arable fields in Canada after the repeated use of the same herbicide for many years.[9] The resistant biotype was about five times more resistant to trifluralin than the susceptible biotype. Resistance is controlled by a single recessive gene.[10] Cross-resistance to other dinitroanilines was also observed.[11,12] This finding provided us with the opportunity to breed foxtail millet for

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trifluralin resistance. Hybrid progenies were obtained after interspecific crosses, and the resistance was shown to be inherited in the crop as two linked recessive genes.[13] As the inheritance pattern in the crop was more complex than in the weed species, it was important to know the extent of resistance of the new germplasm to herbicides of the dinitroaniline group. This information will be useful in determining cost effectiveness and crop safety. This paper reports the response of resistant and susceptible lines to five herbicides of the dinitroaniline group, a class of herbicides that inhibit cell division.

2 EXPERIMENTAL METHODS

2.1 Materials

The resistant plants were derived from a interspecific hybridization between a selected homozygous trifluralin-resistant green foxtail from Oak River, Manitoba (Canada),[9] as the female, and a foxtail millet germplasm 'Amende-4' from Helionkjiang Institute of Crop Breeding, Helionkjiang (China), as the pollen donor. As both green foxtail and foxtail millet are highly autogamous species, crosses were carried out using the technique described by Darmency and Pernès.[2] All the F_1 hybrids were susceptible. The F_2 generation of self-pollination produced 15% of resistant homozygous individuals that were selected for trifluralin resistance in a Petri dish bioassay.[13] Surviving seedlings were planted in pots in the greenhouse, then bagged to produce self-pollinated seed. Twenty of the 130 selected F_2 plants were chosen as they behaved as a typical foxtail millet for plant and seed morphology. They formed 20 F_3 families. The F_3 seedlings were all 100% resistant in the bioassay. The resistant material (R) used here was either the 20 F_3 families, a subset of five F_3 families, or a bulk of these five families. The cv. Amende-4 was used as the susceptible type (S).

2.2 Greenhouse experiments

Plastic trays (45 × 30 × 8 cm) were filled with a mixture of loamy soil and sand (2 + 1 by volume). Herbicides were sprayed directly onto the trays using an automatic sprayer delivering a volume of 500 litre ha^{-1}. Commercial formulations of benfluralin ('Bonalan', Dow Elanco), butralin ('Amex' 820, Sopra), oryzalin ('Surflan', Dow Elanco), pendimethalin ('Prowl' 400, Cyanamid), and trifluralin ('Treflan' EC, Dow Elanco) were applied at the rates listed in Table 1, which correspond to 1/8, 1/4, 1/2, 1 and 2 times the typical field rates recommended for other crops in France,[14] with four replications each. Soil of trays with the same treatment was immediately pooled and carefully mixed before refilling the trays. Each tray was sown with three rows of 20 seeds of R (bulk of five F_3 families) and three rows of S (Amende-4) at 1 cm depth. After sowing, all the trays were sub-irrigated, then distributed at random in the greenhouse (14 h photoperiod and a 25–20°C day/night temperature regime). When untreated seedlings were at the four-leaf stage, 24 days after sowing, the number of emerged seedlings was counted, and the aerial parts were harvested, weighed fresh and again after 72 h at 65°C. Results were expressed as percentage of the untreated control. Linear ($y = a + bx$) and hyperbolic regression analyses ($y = a/(1 + e^{bc}x^{b}) + d$) were carried out using SYSTAT software[15] to calculate the ED_{50} values.

A second experiment used the same procedure but with trifluralin at 1200 g AI ha^{-1} and untreated control only. A replicate consisted in three trays of seven rows each, sown with 30 seeds of each of the 20 R F_3 families and Amende-4. Control and treatments were replicated four times. Emerging seedlings were thinned to give 20

TABLE 1
Effect of Dinitroaniline Herbicides on ED_{50} Values for Emergence Rate and Shoot Dry Matter of S and R Types 24 Days after Treatment in a Greenhouse Experiment

	ED_{50} (g AI ha^{-1})					
	Emergence			*Dry matter*		
Herbicide	*R*	*S*	*R/S*	*R*	*S*	*R/S*
Benfluralin (158 to 2520 g AI ha^{-1})	5100	1200	4	1150	170	7
Butralin (450 to 7200 g AI ha^{-1})	22500	3600	6	4100	500	8
Oryzalin (480 to 7680 AI ha^{-1})	22900	6900	3	400	Nd[a]	Nd[a]
Pendimethalin (165 to 2640 g AI ha^{-1})	5200	1400	4	1160	190	6
Trifluralin (150 to 2400 g AI ha^{-1})	4900	700	7	1360	40	33

[a] Nd: Not determined.

plants in each row. Plant height, fresh and dry weight were measured. Data were analysed by two way ANOVA (factors were doses and family).

2.3 Field experiment

A factorial block design (four replicates) was carried out at the experimental farm of Dijon (France). Plots (3×2 m) were sprayed at 500 litre ha^{-1} with various rates of trifluralin (0, 0·8, 1·2 and 2·0 kg AI ha^{-1}). Immediately following application, the herbicide was incorporated into the 0–10-cm top soil layer using a harrow. Each plot was divided into six sub-plots of 1 m^2. Each sub-plot was hand-sown with 180 seeds of one of five R F_3 families or Amende-4. When untreated plants were at the four-leaf stage, the total number of seedlings was counted, then seedlings were thinned to 60 m^{-2}. Control plots were kept weed-free by hand. Above-ground parts were harvested at the beginning of maturity to avoid biomass loss with seed shedding, because F_3 families were not fixed for that character. Data on emergence, fresh and dry matter for the five R families were averaged for ANOVA comparison with those of the S type. Differences among R families were treated by ANOVA as in the greenhouse experiment 2.

3 RESULTS

3.1 Greenhouse experiments

The lowest dosages of all herbicides had no effect on emergence of the S cultivar, but the highest dosages did, ranging from 50% inhibition (oryzalin) to full inhibition (trifluralin, Fig. 1). The decrease of the emergence rate with increasing doses fitted best a hyperbolic regression model (coefficient of determination $R^2 > 0{\cdot}52$ for all herbicides). In contrast, emergence of the R type was less inhibited and fitted a linear regression model ($R^2 > 0{\cdot}41$). It was always higher than 70%, even at the highest doses. Oryzalin showed the least difference between the two types. The ratio of R versus S ED_{50} values, as calculated from regression equations, ranged from 3 to 7 (Table 1). In addition, the time of emergence of the R type in treated trays was 1·5 days earlier than that of the S type (data not shown).

All dinitroaniline herbicides inhibited seedling growth of both S and R types. Even low doses caused severe stunting of shoots and roots of the S type. Additional injury symptoms were lateral expansion of the meristems. The biomass of the S cultivar decreased with increasing doses, below 20% of that of the control (Fig. 2). The data fitted the hyperbolic model ($R^2 > 0{\cdot}67$). In the case of oryzalin, high inhibition was found at all doses, and it has not been possible to estimate the ED_{50} value because it was out of the range of doses tested. Seedlings of the R type also showed these symptoms but at higher doses, and data also fitted hyperbolic regression equations ($R^2 > 0{\cdot}68$). Oryzalin was the most effective herbicide on both the S and R types. For other herbicides, the R/S ratio ranged from 6 to 33, the highest difference between S and R types being observed with trifluralin (Table 1). Data for fresh weight (not shown) provided similar results.

Experiment 2 showed that treatment with trifluralin at 1200 g ha^{-1} significantly reduced the biomass of the R plants, in a similar way to that in experiment 1 (Table 2). Significant differences of height and fresh weight were observed among families. However, treatment × family interaction of the two-way ANOVA was not significant, indicating that the treatment had not changed the relative performances of the F_3 families. Therefore, segregation of characters in the F_2 generation resulted in F_3 families having different growth, but all families

TABLE 2
Effect of Trifluralin at 1200 g AI ha^{-1} on Height, Fresh and Dry Weight per Plant of Twenty Resistant Families in Greenhouse

		Height (*cm*)	*Fresh weight* (*mg*)	*Dry weight* (*mg*)
Mean	Control	10·6	85	8·5
	Treated	7·7	35	5·7
Range	Control	3·7–14·5	17–142	3·4–13·1
	Treated	3·0–13·0	13–94	2·3–11·0
2-way ANOVA[a]	Treatment	***	***	***
	Family	*	***	NS
	Dose × Fam	NS	NS	NS

[a] NS, *, *** not significant at 5% level, significant at 5% and 0·1% levels, respectively.

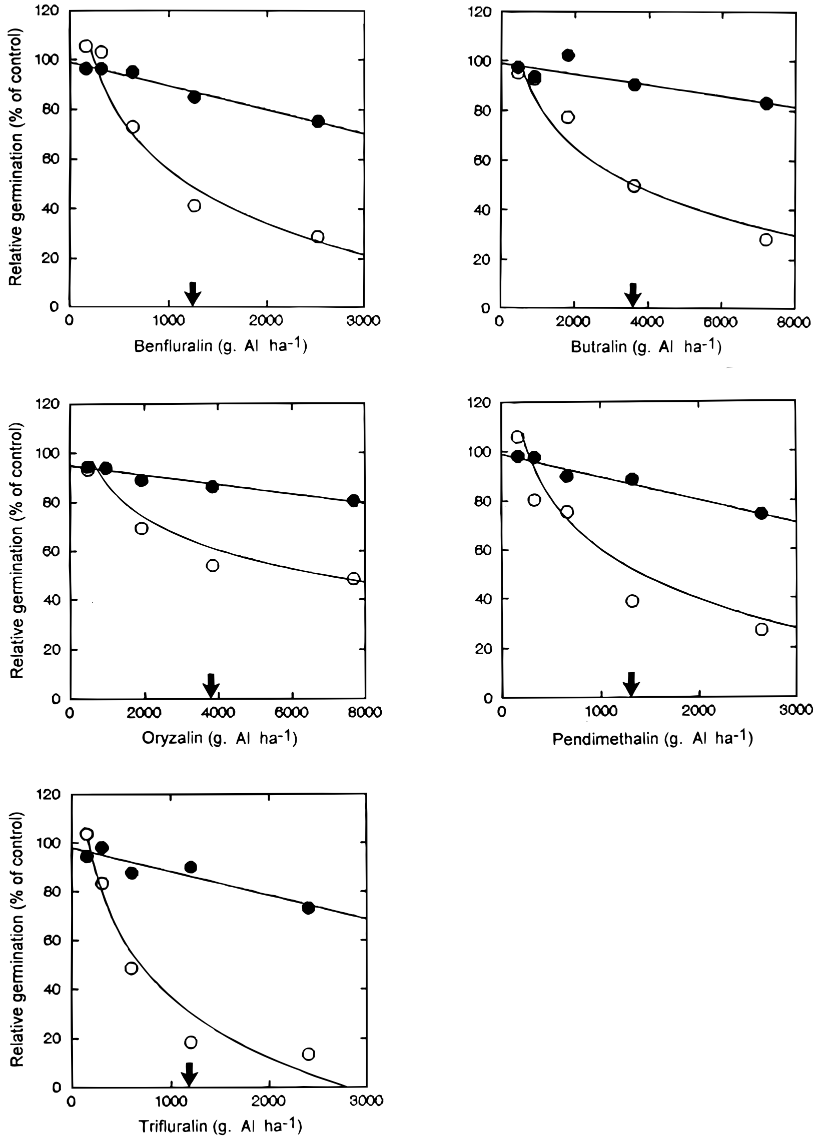

Fig. 1. The effect of increasing doses of benfluralin, butralin, oryzalin, pendimethalin and trifluralin on the emergence of (○) S and (●) R types of foxtail millet. Arrows denote typical recommended field rates.

TABLE 3
Germination and Biomass of S and R Types in a Field Experiment with Soil-Incorporated Trifluralin[a]

Rate ($g\ AI\ ha^{-1}$)	*Type*	*Emergence* (%)	*Fresh weight* (*g per plant*)	*Dry weight* (*g per plant*)
0	S	59·9 a	33·1 a	12·7 a
	R	58·0 a	23·7 b	9·7 b
800	S	0	0	0
	R	34·2 b	19·9 c	8·6 c
1200	S	0	0	0
	R	22·2 c	Nd[b]	Nd
2000	S	0	0	0
	R	11·5 d	Nd	Nd

[a] Same letters in a column indicate the values are not significantly different at 1% level.
[b] Nd = Not determined.

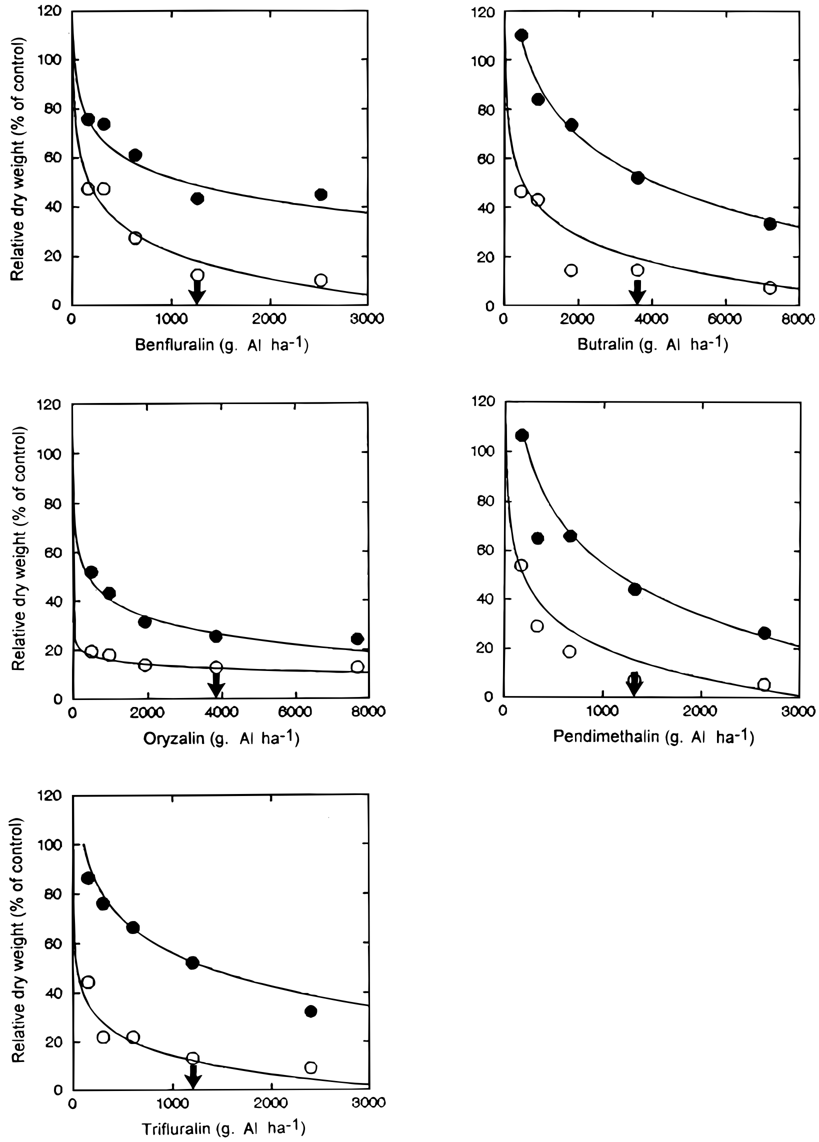

Fig. 2. The effect of increasing doses of benfluralin, butralin, oryzalin, pendimethalin and trifluralin on the shoot dry matter production of (○) S and (●) R types of foxtail millet. Arrows denote typical recommended rates.

behaved similarly in response to the herbicide treatment.

3.2 Field experiment

There was no difference in the extent of emergence of the S and R types in untreated field plots. At the rate of 800 g AI ha^{-1} and higher, the S type did not emerge, while the emergence rate of the R type was somewhat reduced (Table 3). The ED_{50}, calculated from the best fitted regression equation ($R^2 = 0{\cdot}92$), was nearly 900 g AI ha^{-1}. At higher doses, the emergence of the R type was too low to result in a 60 plant m^{-2} density, so that the biomass measurements were not carried out. The S type had significantly higher biomass than the R type in control conditions. The R type treated at 800 g ha^{-1} showed 85% of the fresh and dry weights of the control, which was a significant decrease at the 1% level (Table 3). Significant differences of fresh and dry weight were found among R F_3 families, both in treated and control conditions (not shown). As in experiment 2, no dose × family interaction was detected, indicating that the plant response to the herbicide was of the same magnitude whatever the family.

4 DISCUSSION

The resistance genes transferred to the cultivated foxtail millet resulted in increased resistance to all dinitroaniline herbicides. The R/S ratio, calculated as ED_{50}, ranged from 3 to 33 according to herbicide and whether

measured as emergence rate or as biomass. The highest R/S ratios were recorded for trifluralin, especially for biomass, and the lowest for oryzalin. Results obtained on studies of green foxtail provided similar resistance ratios.[11,12] Oryzalin is the most water-soluble and the most volatile chemical of the dinitroanilines used here, which might account for more intense effects in small tray tests in the greenhouse. In addition, pre-plant incorporated treatment (ppi) is not necessary for all chemicals. If ppi is necessary for trifluralin and benfluralin, it is only recommended in some crops for pendimethalin, and is not necessary for butralin and oryzalin.[14] Thus, although trifluralin appeared to be the most effective herbicide in the greenhouse test, this did not preclude the use of the other chemicals in fields trials under the current mode of application. Other patterns of cross-resistance were found in species having different mechanisms or inheritance of the dinitroaniline resistance, but R/S ranged from 3 to 15 in all cases, which denoted moderate resistance.[16–19]

Breeding for herbicide resistance in crops must focus not only on the R/S ratio, but especially on the absolute value of the resistance to prevent detrimental effects on the crop. Satisfactory emergence was achieved in the greenhouse for the R type at most doses of all herbicides. However, only 38% of potential emergence was obtained in the field trial with trifluralin at 1200 g ha^{-1}. Even the emergence rate in untreated controls in the greenhouse was different from that in the field experiment, indicating that care must be taken in extrapolating results of greenhouse experiments to field conditions, probably because of more complex and less homogeneous soil conditions. In order to correct the reduced emergence rate in the field, one could increase the seed sowing density to provide the final crop density in the field. Also, one can imagine using seed coating with antidote to best protect resistant plants against herbicide injury, as achieved by dinitroaniline protectant in sorghum (Molin, W., pers. comm.), a crop belonging to the same botanical family as millet.

The herbicide effect at the seedling stage was greater than at emergence, as ED_{50} values were at least five times lower. There is no possibility of getting higher resistance from the present plant material as no significant difference in herbicide resistance was detected among F_3 families. Growth inhibition occurred also at 800 g ha^{-1} trifluralin in the field, although it was less pronounced than in the greenhouse. Roots of adult foxtail millet plants could escape the trifluralin-contaminated area so that fewer detrimental effects were observed in the field in comparison to seedling trials in small trays in the greenhouse. At the recommended field dose of trifluralin, 1200 g ha^{-1}, a dose which provides good weed control in other crops in France,[14] both emergence and growth of the R lines are too inhibited to use this plant material in commercial fields. However, a 800 g ha^{-1} dose completely killed the S type while it had no effect on emergence and only 12% growth inhibition effect on the R type. This dose also kills 99% of green foxtail populations.[20] As green foxtail is the most important weed infesting foxtail millet fields, this dose could be used for weed control. The five dinitroaniline herbicides tested here control grass weeds, including barnyardgrass, goosegrass, green foxtail and certain broadleaf weeds such as redroot pigweed, so that they appear to be well suited for use in foxtail millet fields in China as well as in Europe. On the other hand, plant plasticity at adult stage perhaps could compensate for weak seedling damage due to 800 g ha^{-1} trifluralin and result in normal grain yield. Further testing of these observations must be carried out with improved germplasms in foxtail millet production areas. Besides, if the resistance is transferred into male sterile lines, it could be very useful to preserve line purity and homogeneity from seed or pollen contamination, which is a big problem now for breeders,[21] because any heterozygous plant is susceptible and can be killed by low doses of trifluralin. Weed-control programs, including rotating herbicides to prevent the occurrence of herbicide weed resistance through mutation and crop × weed spontaneous interspecific hybridization, must also be studied.

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